Journal of the American College of Cardiology
Vol. 51, No. 6, 2008

Published by Elsevier Inc.
ISSN 0735-1097/08/$34.00
doi:10.1016/j.jacc.2007.09.057

EDITORIAL COMMENT

New Insights on Endothelial Progenitor Cell Subpopulations and Their Angiogenic Properties*

Eduard Shantsila, MD,†
Timothy Watson, MRCP,† Hung-Fat Tse, MD,‡
Gregory Y. H. Lip, MD†

Birmingham, United Kingdom; and Hong Kong, China

In 1997, Asahara et al. (1) reported the isolation of putative endothelial progenitor cells (EPCs) from human peripheral blood, on the basis of cell surface expression of both hematopoietic stem cell (CD34) and kinase insert domain receptor (KDR) markers. They observed that these cells differentiate into endothelial cells in vitro and in vivo. These cells have opened new perspectives for "regenerative" cardiological interventions, and EPCs have since attracted much interest with many studies demonstrating strong association between the quantity/function of such cells and different aspects of cardiovascular health (2–4).

See page 660

Enthusiasm for EPCs needs to be tempered by definition of what EPCs are, as well as the availability of a reliable method to assess their quantity and quality, functional status, and therapeutic application. In particular, neither is CD34 a specific stem cell marker nor is KDR a specific mature endothelial cell marker (Fig. 1). The search for more specific and reliable markers of "true" EPCs is therefore ongoing. Consequently, an early hematopoietic stem cell marker, CD133, was adopted as an alternative additional marker to indicate "true" EPCs (5). The CD133 marker is expressed on hematopoietic stem cells and progenitor cells from human bone marrow, fetal liver, and peripheral blood (6). In culture, these cells grow endothelial cell colony-forming units (CFU-ECs), which are also referred to as "early endothelial colonies," with rapid down-regulation of CD133 and acquisition of some mature endothelial markers (5). It was therefore proposed that CD133 might provide a more reliable means of defining and tracking human angioblast-like EPCs and distinguishing these from mature endothelial or monocytic cells. As a result, the combination phenotype of $CD34^-/CD133^+/KDR^+$ is now commonly used as a definition for EPCs.

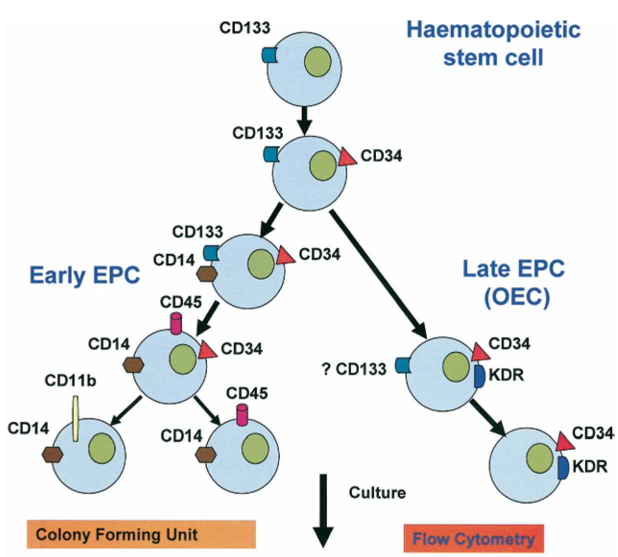
Nonetheless, EPC subpopulations may well display different angiogenic properties. As highlighted in this issue of the *Journal*, Sieveking et al. (7) provide new insights from a novel human angiogenesis assay, showing that although fresh EPCs can be directly incorporated into the endothelial monolayer, other endothelial-like cells from CFU-ECs, when transplanted into ischemic tissues, they promote angiogenesis and are found around the neovasculature but are not incorporated within it (7). These spindle-like cells appear to possess a relatively low proliferative capacity and a low ability to express mature endothelial proteins (8). The exact mechanisms of their effects on cardiovascular health are still poorly understood, with no evidence that these cells are a major source of mature endothelial cells.

These important observations may make some previous study results difficult to interpret, given that more than 1 kind of cell with angiogenic properties exist. This raises some controversy with respect to the identification and origin of isolated EPCs, because these cells seem to reflect a functional subpopulation within the blood mononuclear cells that have the potential to differentiate into an endothelial phenotype in vivo.

As shown in Figure 1, the definition of EPCs by surface markers is challenging, given the change in surface marker profiles during the process of mobilization and maturation. For example, $CD34^-/133^+$ progenitors differentiate into $CD34^+/133^+$ EPCs that possess more pronounced angiogenic properties (9). Of note, no correlation was found between the number $CD34^+$ or $CD133^+$ circulating cells and the numbers of CFU-ECs (10). Moreover, erythrocyte lysing before flow cytometry affects cell survival and has a high impact on the quantitative analysis of EPCs, with 20% lower numbers compared with nonlyse protocols (11). In addition, other sorts of endothelial colonies can be grown. For example, there are at least 2 morphologically and functionally distinct endothelial cell populations that can probably be grown from circulating mononuclear cells: the so-called "early" and "late" EPCs according to the timing of their emergence during culture. The early EPCs form the more conventional CFU-ECs, and late EPCs are thought to represent those "outgrowth" endothelial cells (OECs) as described by Sieveking et al. (7). Thus, the quantity and functional evaluation of putative endothelial progenitors may be problematic, owing to the low specificity of cell culture techniques.

*Editorials published in the *Journal of the American College of Cardiology* reflect the views of the authors and do not necessarily represent the views of *JACC* or the American College of Cardiology.

From the †Haemostasis, Thrombosis and Vascular Biology Unit, University Department of Medicine, City Hospital, Birmingham, United Kingdom; and the ‡Cardiology Division, Department of Medicine, University of Hong Kong, Hong Kong, China.



Early EPC	Characteristics	Late EPC
3-5 days	Duration	7-14 days
Heterogeneous	Morphology	Homogenous
$CD14/CD45/KDR_{Low}$ CD31/vWf	Antigen markers	CD34/KDR CD31/vWf
+++	Secretion of angiogenic cytokines	+
Low	*In-vivo* angiogenic effects	High

Figure 1 **Properties and Antigenic Cell Surface Markers of Both "Early" EPCs and "Late" EPCs (OECs)**

Note that continual maturation results in antigenic marker shift, and therefore identification of subpopulations has often proved to be challenging. Also note that that current endothelial progenitor cell (EPC) definitions are based predominantly on phenotype. Early EPCs are, however readily identifiable by their ability to form colony-forming units on cell culture, whereas late EPCs are more readily identifiable using flow cytometry. As cells continue to mature and integrate into the endothelium, expression of CD31 and von Willebrand factor (vWf) increases. OEC = outgrowth endothelial cell; KDR = kinase insert domain receptor.

What Are OECs?

The EPCs growing into OECs show high proliferative potential and originate predominantly from bone marrow donors. These are considered to be circulating angioblasts, although relatively few studies have fully characterized their properties. The so-called "true" EPCs as estimated by flow cytometry (i.e., $CD34^+/CD133^+/KDR^+$ cells) yield the OECs during cell culture. As shown in Figure 1, formation of OEC colonies has been exclusively found in the $CD34^+/CD133^-$ selected cell population but not the $CD34^-/CD133^+$ or $CD34^+/CD133^+$ populations (12). The leukocyte/monocyte antigens CD45 and CD14 were also not detectable in separated $CD34^+/CD133^-$ cells. In another study, only the small BM $CD34^+/CD45^-$ cell fraction, but not the $CD34^+/CD45^+$ hematopoietic progenitor fraction nor any other $CD45^+$ subpopulation, were able to grow OECs (13). Furthermore, OECs generating $CD34^+/CD45^-$ cells express KDR but not CD133, whereas $CD34^+/CD45^+$ hematopoietic progenitors express CD133 (13).

In contrast, early EPCs are considered to represent cells of probable different lineage, which includes a subset of $CD14^+/CD34^-$ monocytic cells with the potential to differentiate into endothelial-like cells and form CFU-ECs under certain environmental conditions (14). The available data suggest that CFU-ECs belong to the monocyte-macrophage lineage, because they share many immunophenotypic and molecular features (15). In polycythemia rubra vera patients harboring a Janus kinase 2 V617F mutation in hematopoietic stem cell clones, for example, CFU-ECs, were shown to possess myeloid progenitor cell activity, to differentiate into phagocytic macrophages and to fail to form perfused vessels in vivo, strikingly in contrast to OECs, which are clonally distinct from CFU-ECs (16).

What Other Evidence Is There for Subtypes of EPCs?

When a highly sensitive antibody-conjugated magnetofluorescent liposomes technique was used, Romagnani et al. (17) found a population of $CD14^+/CD34^{low}$ cells, which represented a variable proportion of $CD14^+$ cells and constituted the dominant population among circulating KDR^+ cells. In that study, virtually all $CD14^+$ cells present in the bone marrow were found to be $CD14^+/CD34^{low}$ double-positive cells.

Are These Differences Important?

Although monocyte-derived EPCs have a lower in vitro proliferation potential than hematopoietic stem cells or cord-blood–derived EPCs, the different progenitor types seem to have a similar ability to enhance neovascularization in experimental models (18). One may speculate that proliferative capacity is not the only decisive factor and that the monocyte-derived EPCs are also likely to play an important role in angiogenesis and arteriogenesis via a paracrine effect with the release of growth factors, such as vascular endothelial growth factor, as shown by Sieveking et al. (7).

However, when human $CD34^+$ cells or $CD14^+$ cells were locally delivered into the ischemic limbs of diabetic mice, $CD14^+$ cell therapy improved healing and vessel growth more slowly and not as effectively as $CD34^+$ progenitors (19), perhaps because $CD34^+$ cells also include OEC populations, which (as previously discussed) appear to have more angiogenic potential. Different cell populations might also affect the function of each other, and their use in combination as "angiogenic therapy" may be more effective than using isolated populations alone (20). Of note, the presence of a $CD34^+/CD133^+/CD14^+$ subpopulation of circulating EPCs has recently been demonstrated in peripheral blood (12).

Future Directions

The EPCs first described by Asahara et al. (1) that have attracted the most attention among researchers are essentially monocyte-derived hematopoietic $CD14^+$ cells with variable expression of CD34, CD133, CD45, and KDR, which can form CFU-ECs and differentiate into macrophages (12,21). These cells do not incorporate into the vascular endothelial monolayer but still support angiogenesis. The current study by Sieveking et al. (7) is important and timely, given that it elegantly demonstrates—with a novel human EC-specific angiogenesis assay—that these kind of cells do not directly participate in the formation of new vascular-like structures but strongly augment it in a paracrine fashion.

The value of single or complex markers for different EPC populations still remains controversial and clearly reflects the heterogeneous nature of endothelial precursors. Even though it is now known that OECs are derived from a small $CD34^+/CD133^-/CD45^-/CD14^-$ population, no strong surface marker definition of OECs currently exists. As a result, marker estimations and culture properties have to be used by many researchers to define EPCs. Therefore, when functional properties of EPCs are evaluated in vitro, more specific approaches, such as the one proposed by Sieveking et al. (7), should be employed.

Given novel data of morphology and biological properties of different EPC subpopulations (22,23), the results of earlier studies perhaps should be carefully re-examined, and the impact of these subpopulations have to be taken into consideration when futures studies are performed. The types of cells studied or used for such therapy have to be clearly characterized to avoid misleading results. Possible angiogenic properties of EPCs should also be confirmed by in vivo or in vitro angiogenic models. Clearly, the precise biological role(s) of different EPCs alone or in combination require further clarification to open new avenues for cardiovascular therapy. We live in exciting times.

Reprint requests and correspondence: Prof. Gregory Y. H. Lip, City Hospital NHS Trust, University Department of Medicine, City Hospital, Dudley Road, Birmingham B18 7QH, United Kingdom. E-mail: g.y.h.lip@bham.ac.uk.

REFERENCES

1. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997;275:964–7.
2. Lau KK, Chan YH, Yiu KH, Li SW, Tam S, Lau CP, Kwong YL, Tse HF. Burden of carotid atherosclerosis in patients with stroke: relationships with circulating endothelial progenitor cells and hypertension. J Hum Hypertens 2007;21:445–51.
3. Boos CJ, Goon PK, Lip GY. Endothelial progenitor cells in the vascular pathophysiology of hypertension: arterial stiffness, ageing and more. J Hum Hypertens 2006;20:475–7.
4. Shantsila E, Watson T, Lip GY. Antioxidant protection: yet another function of endothelial progenitor cells? J Hum Hypertens 2007;21:343–6.
5. Peichev M, Naiyer AJ, Pereira D, et al. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. Blood 2000;95:952–8.
6. Yin AH, Miraglia S, Zanjani ED, et al. AC133, a novel marker for human hematopoietic stem and progenitor cells. Blood 1997;90:5002–12.
7. Sieveking DP, Buckle A, Celermajer DS, Ng MKC. Strikingly different angiogenic properties of endothelial progenitor cell subpopulations: insights from a novel human angiogenesis assay. J Am Coll Cardiol 2008;51:660–8.
8. Hur J, Yoon CH, Kim HS, et al. Characterization of two types of endothelial progenitor cells and their different contributions to neovasculogenesis. Arterioscler Thromb Vasc Biol 2004;24:288–93.
9. Friedrich EB, Walenta K, Scharlau J, Nickenig G, Werner N. CD34−/CD133+/VEGFR-2+ endothelial progenitor cell subpopulation with potent vasoregenerative capacities. Circ Res 2006;98:e20–5.
10. Tura O, Barclay GR, Roddie H, Davies J, Turner ML. Absence of a relationship between immunophenotypic and colony enumeration analysis of endothelial progenitor cells in clinical haematopoietic cell sources. J Transl Med 2007;5:37.
11. Rustemeyer P, Wittkowski W, Greve B, Stehling M. Flow-cytometric identification, enumeration, purification, and expansion of CD133+ and VEGF-R2+ endothelial progenitor cells from peripheral blood. J Immunoassay Immunochem 2007;28:13–23.
12. Untergasser G, Koeck R, Wolf D, et al. CD34+/CD133− circulating endothelial precursor cells (CEP): characterization, senescence and in vivo application. Exp Gerontol 2006;41:600–8.
13. Timmermans F, Van Hauwermeiren F, De Smedt M, et al. Endothelial outgrowth cells are not derived from CD133+ cells or CD45+ hematopoietic precursors. Arterioscler Thromb Vasc Biol 2007;27:1572–9.
14. Harraz M, Jiao C, Hanlon HD, Hartley RS, Schatteman GC. CD34(−) blood-derived human endothelial cell progenitors. Stem Cells 2001;19:304–12.
15. Lopez-Holgado N, Alberca M, Sanchez-Guijo F, et al. Short-term endothelial progenitor cell colonies are composed of monocytes and do not acquire endothelial markers. Cytotherapy 2007;9:14–22.
16. Yoder MC, Mead LE, Prater D, et al. Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. Blood 2007;109:1801–9.
17. Romagnani P, Annunziato F, Liotta F, et al. $CD14^+CD34^{low}$ cells with stem cell phenotypic and functional features are the major source of circulating endothelial progenitors. Circ Res 2005;97:314–22.
18. Urbich C, Heeschen C, Aicher A, Dernbach E, Zeiher AM, Dimmeler S. Relevance of monocytic features for neovascularization capacity of circulating endothelial progenitor cells. Circulation 2003;108:2511–6.
19. Awad O, Dedkov EI, Jiao C, Bloomer S, Tomanek RJ, Schatteman GC. Differential healing activities of CD34+ and CD14+ endothelial cell progenitors. Arterioscler Thromb Vasc Biol 2006;26:758–64.
20. Suuronen EJ, Wong S, Kapila V, et al. Generation of CD133+ cells from CD133− peripheral blood mononuclear cells and their properties. Cardiovasc Res 2006;70:126–35.
21. Rohde E, Bartmann C, Schallmoser K, et al. Immune cells mimic the morphology of endothelial progenitor colonies in vitro. Stem Cells 2007;25:1746–52.
22. Tao J, Wang Y, Yang Z, Tu C, Xu MG, Wang JM. Circulating endothelial progenitor cell deficiency contributes to impaired arterial elasticity in persons of advancing age. J Hum Hypertens 2006;20:490–5.
23. Yang Z, Wang JM, Chen L, Luo CF, Tang AL, Tao J. Acute exercise-induced nitric oxide production contributes to upregulation of circulating endothelial progenitor cells in healthy subjects. J Hum Hypertens 2007;21:452–60.